NTP
National Toxicology Program

Toxicogenomics at the National Toxicology Program

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NTP Board of Scientific Counselors Meeting

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Toxicogenomics faculty

- Composition:
  - scientists from NTP, NIEHS, outside organizations where appropriate
- Responsibilities
  - Develop and execute a strategy for implementation of toxicogenomics
  - Development, review, and approval of research proposals
  - Serve as a resource for NTP scientists

Current areas of investigation

- Basic issues that impact the design and interpretation of toxicogenomic studies
  - Extent of “normal” variation of animal models under typical study conditions
    - Animal husbandry: housing, type of feed, etc.
    - Study length
    - Circadian rhythm
    - Estrous cycle in females
    - Age related effects
    - inter-laboratory variation
  - Liver (hepatic transcriptome)
- As a complement to standard toxicology/carcinogenesis studies to aid in understanding toxic mechanisms and providing information for dose selection
- Identification of gene expression patterns associated with cancer
  - hepatocarcinogenicity

Completed studies

- Sources of variablity in toxicogenomic studies
- The use of machine learning and prechronic gene expression patterns to classify hepatocarcinogens and non-carcinogens

Sources of variability in toxicogenomic studies

- Variability among individual animals
- Variability associated with the normal circadian cycle
- Variability associated with ageing

Animal to animal variability

Does basal gene expression in untreated male F344 rats, of the same age, and maintained under conditions specified for NTP toxicology studies, exhibit statistically significant differences?

- 3-day time period was selected to approximate the time required for a prechronic necropsy
- Animals (6/group) necropsied 6, 18, 24, and 48 hrs after single gavage dose of 0.5% aqueous methylcellulose
- Individual body weights, clinical chemistry, histopathology, hepatic glutathione
- Hepatic transcript profile

Results

- Detectable animal to animal variation in transcript profiles of in-bred animals is present under standard NTP laboratory conditions
- No single transcript was differentially expressed (expression ratio significantly different from 1.0) in all 24 animals; only 7 transcripts are differentially expressed in any 10 of the 24 animals
- Transcript profiles show no obvious association to normal variation in standard toxicological parameters of time matched controls

Conclusion

•Basal gene expression in untreated male F344 rats, of the same age, and maintained under conditions specified for NTP toxicology studies, does not exhibit statistically significant differences

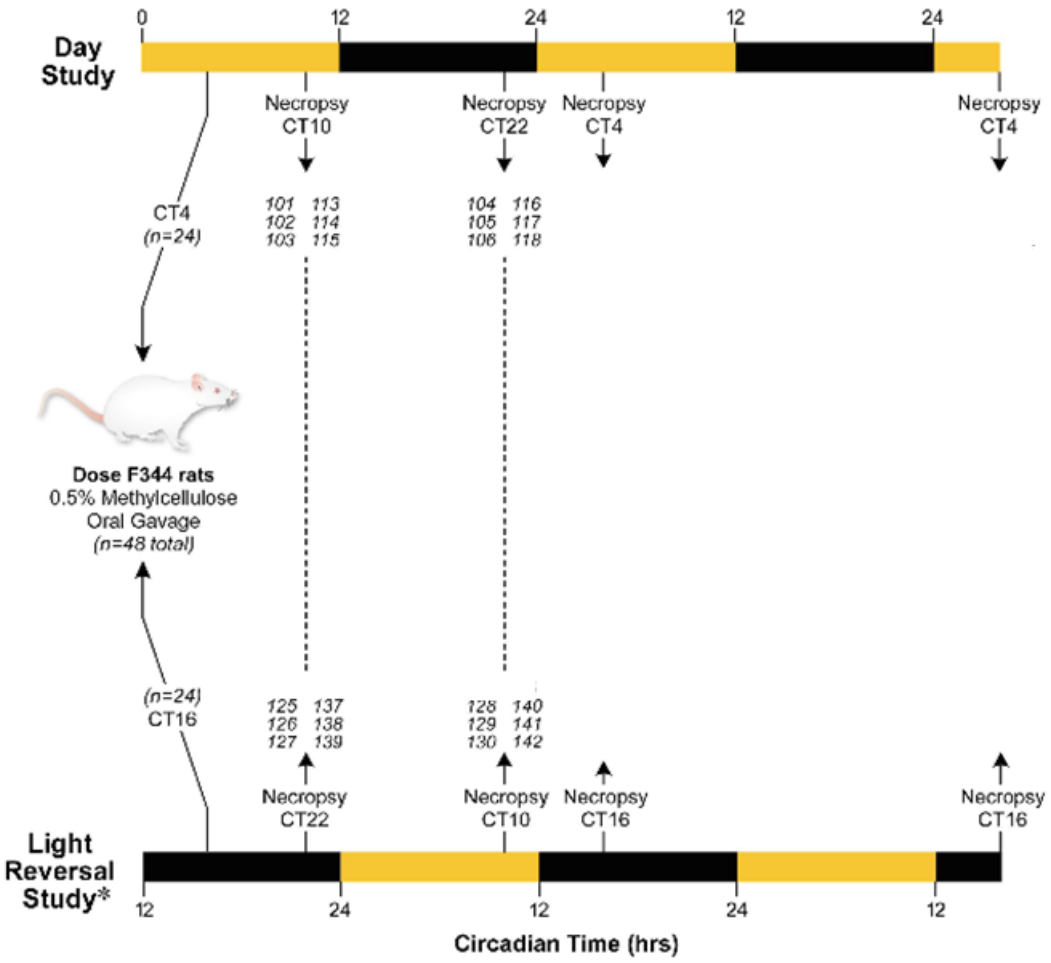
• Necropsy conditions are well controlled, uniform, and reproducible within a study

The effect of the circadian cycle on the hepatic transcriptome

Given: Circadian rhythm effects the hepatic transcriptome

- How significant is the impact of the circadian cycle over a typical 3-day necropsy?
- What transcripts are involved?

Circadian experimental design



Analysis of 12-hour offset data

CT-10 (day) individuals hybridized against pooled RNA from CT-22 (light reversal) animals

CT-22 (light reversal) individuals hybridized against pooled RNA from CT10 (day) animals

Similar analysis for CT-4 and CT-16 animals

972 transcripts in common including transcripts for clock genes, clock control genes, and genes involved with intermediary metabolism

Fourier analysis

Given: Transcripts of genes involved with the circadian cycle exhibit a cyclic pattern of expression over each 24 hour circadian cycle

- Pooled RNA from each of the four time points (CT-4, CT-10, CT-16, CT-22) was hybridized to a universal reference standard
- Fourier transform as implemented by Whitfield et al (2002) was used to identify transcripts whose levels varied periodically over the four time points
- Identified 1300 transcripts; 200 in common with the 12-hour offset list

Conclusions

- Circadian rhythm has pronounced effect on hepatic transcriptome
- To control for circadian effects, the design of toxicogenomic studies must specify that groups of animals to be compared e.g. treated vrs. control, shall be necropsied within a 3-4 hour time window of each other

Age related changes of the hepatic transcriptome in sentinel animals from NTP 2-year studies

Given: Age has a significant impact on the hepatic transcriptome of male F344 rats

- Sentinel male F344 rats 32, 58, or 84 weeks of age (6, 12, or 18 months of age) from Battelle Columbus
- RNA from Individual animals hybridized against a pool consisting of equal amounts of RNA from all 63 rats

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Conclusions

- Significant age related changes occur in hepatic transcriptome
- Essential to use age (days-week) and time (hours) matched controls
- Biotransformation and metabolite profiles of chemicals may change with aging

Impact of the estrous cycle on hepatic transcriptome of female rats

Given: The estrous cycle has a significant impact on the hepatic transcriptome in female animals

- To be able to compare treated and control *female* animals, it is necessary to identify those transcripts which are significantly impacted by the estrous cycle and the time profile of those changes to be able to distinguish treatment related changes from those associated with cycling
- This will determine the relative impact of different stages of the estrous cycle on the hepatic transcriptome and provide a basis for determining the appropriate stage and time window during which treated and control animals must be necropsied
- This will also provide a basis for interpretation of sex differences in response to chemical exposures in the liver

Microcystin class study

- Microcystin
  - Widespread algal toxin class (>60 members)
  - Microcystin-LR one of the most common toxins in surface water
  - Total “LR equivalents” used for water standards
- Available only in limited quantities so conventional multi-dose toxicology studies not feasible
- Single dose studies comparing hepatic transcript profiles of different microcystins
- Hypothesis
  - Different microcystins and microcystin mixtures exhibit similar hepatic toxicity (based on pathology, clinical chemistry, toxicogenomics) at comparable “LR equivalent” doses

Studies with a toxicogenomic component

- Tissue will be collected for possible transcript profiling in several prechronic studies
  - Aminopyridines
  - Caffeine+ephedrine - *heart*
  - 2-hydroxy-4-methoxybenzophenone
  - 2-methoxy-4-nitroaniline
  - Pentabromodiphenyl oxide (DE71)
  - Class study of perfluorinated chemicals
- Toxicogenomics included on a case by case basis

Future plans

- Continue current activities
  - examination of basic sources of variation
  - Expand data base of prechronic data available for machine learning and refine classification models
- Employ greater use of toxicogenomic end points in understanding and interpretation of toxic responses
  - Greater mechanistic understanding
  - More refined dose selection
- Improved hazard identification